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(54) Title: *IN VITRO* DIAGNOSIS METHOD FOR EARLY DETECTION OF CERVICAL DYSPLASIAS AND CANCER, ASSOCIATED WITH HPV

(57) Abstract: The present invention relates to in vitro diagnosis method for early detection of cervical dysplasias and cervical cancer, associated with human papilloma viruses. The method claimed covers identification and quantity determination of oncoprotein E7 HPV (types 16 and 18), which is considered a biochemical marker of malignization of epithelial cells by using two types - a couple of enzyme-labeled monoclonal antibodies and provides a reliable proof of the primary stage of neoplastic transformation of cervical cells. The couple of enzyme-labeled monoclonal antibodies are type 716-281 and type 716-332. Due to a large excess of enzyme-labeled monoclonal antibodies used the range of sensitiveness for detection of oncoprotein E7 HPV types 16 and 18 is extended from 40 pg/ml to 50 ng/ml.

IN VITRO DIAGNOSIS METHOD FOR EARLY DETECTION OF CERVICAL DYSPLASIAS AND CANCER, ASSOCIATED WITH HPV

FIELD OF THE INVENTION

The present invention relates to *in vitro* diagnosis method for early detection of cervical dysplasias and cervical cancer associated with human papilloma viruses.

BACKGROUND OF THE INVENTION

The papilloma virus infection is one of the most widespread viral infections. Over 50% of sexually active population all over the globe becomes infected with the human papilloma virus (HPV) during the lifetime. HPV infection is the cause of various genital disorders, such as cervical, vulvas, vaginal and anal carcinomas, genital condylomas (Kiselev V.I., Dmitriev G.A., Kubanova A.A. Interrelationship between sex-transmitted viral infections and urogenital cancers. Vestnik Dermatologii i Venerologii (Bulletin of Dermatology and Venerology), 2000, No. 6, 20-23 (in Russian). (For illustration, see Fig. 1).

In the overwhelming majority of patients, HPV infection disappears completely and culminates in spontaneous recovery. However, in ~ 10% of cases this infection takes a more chronic course.

Cervical carcinoma is the second widespread oncological disease in women. Every year, this disease affects ~ 500000 females all over the globe. The incidence of HPV-associated rectal carcinomas has increased twofold in the past 25 years. However, the screening programs aimed at early detection of the disease are often inefficient, being sporadic and non-informative.

Laboratory diagnosis

Laboratory diagnosis of papilloma virus infection specifically directed at early detection of virus carriers is a means of control over the spreading of HPV infection aimed at timely implementation of preventive and therapeutic measures (combined therapy) against the development of neoplasm's.

Routine diagnostic procedures used in the clinical practice over many decades usually employ cytological tests (the so-called Pap-smears) and allow the detection of atypical

mononuclear cells. However, many authors believe that this procedure detects no more than 30% infection carriers and is out of date.

In developing reliable diagnostic procedure for early detection of cervical dysplasias serological analysis enabling to detect antibodies to HPV oncoproteins in patient's serum was used (see patent EP 0406542 *A method for detecting precancerous and cancerous cervical intraepithelium* and patent EP 0523391 *Use of HPV-16 E6 and E7-gene derivated peptides for diagnostic purpose*). The patents disclose detection of the specific antibodies by immunoenzymatic and radio-immunE assays using peptide determinants of HPV proteins. However, the method mentioned above failed due to absence of reliable correlation between the levels of antibodies and cervical cancer.

Typing of HPV using the polymerase chain reaction (PCR) has become very popular in recent years. This method has a high diagnostic potential and enables identification of individual types of HPV; see, for example В. И. Киселев, Г. А. Дмитриев, М. Ф. Латыпова. Полимеразная цепная реакция в диагностике урогенитальных инфекций. Пособие для врачей. Москва. 2000; U. Wieland, H.Pfister "*Molecular diagnosis of persistent human papilloma virus infections*". Intervirology, 1996, v.39, pp. 145-157; WO 00506645 *Method and KIT for early cancer prediction*; US 5679509 *Methods and diagnostic aid for distinguishing a subset of HPV that is associated with an increased risk of developing cervical cancer dysplasia and cervical cancer.*

However, more recent studies into the mechanisms of HPV infection have demonstrated that the infectious process includes two stages, viz., reproductive proliferation of the virus (stage 1) and integrative infection where the virus DNA is incorporated into the genomes of infected cells (stage 2).

The first stage is reversible and often leads to spontaneous remission. The integrative infection stage is the first step to neoplastic transformation of cells which stimulates malignant growth (R.W. Tinkle "Immune evasion in human papillomavirus-associated cervical cancer". Nature reviews, 2002, v.2, pp1-7).

However, PCR-based diagnosis does not discriminate between these two stages and positive results of PCR analyses do not always allow prediction of malignant processes.

Recently, it has been found that integration of HPV DNA into the cell genome is accompanied by a vigorous synthesis of viral oncoproteins, of protein E7, in particular. According to the generally accepted viewpoint, the presence of protein E7 in cervical

samples is an unequivocal proof of malignization of epithelial cells containing an integrated copy of the HPV genome. The statement above is confirmed by the results presented in patent WO 0154713 *Method for determining risk of developing cervical cancer*. However, the immunohistochemical method used therein for detection of onco-proteins E6 and E7 in cervical bioptats is inferior to immunoenzymatic method developed by us.

DESCRIPTION OF THE INVENTION

The present invention aims to provide for reliable *in vitro* diagnosis method for early dysplasias and cervical cancer associated with human papilloma viruses. The task is realized by quantity determination of oncoprotein E7 HPV (types 16 and 18) by using two types – a couple of enzyme-labeled monoclonal antibodies. The couple of enzyme-labeled monoclonal antibodies used are type 716-281 and type 716-332 and they are used in a large excess. The method claimed allows to detect oncoprotein E7 HPV types 16 and 18 in the range of concentrations from 40 pg/ml to 50 ng/ml.

BRIEF DESCRIPTION OF THE FIGURES

Fig. 1. *The development of a papilloma virus infection.*

Fig. 2. The structure of the genetic conjugate (plasmid) is shown.

Fig. 3. Synthesis of oncoproteins E7 HPV-16 and E7 HPV-18 in *E.coli* cells

Fig. 4. A calibration curve for quantitation of protein E7 HPV16 in a sandwich-EIA test. MoAb 716-288 were adsorbed from 0.1M carbonate buffer pH 9.6 (5 μg/ml). The working dilution of the MoAb 716-332 – peroxidase conjugate was 1/50000 in PBS containing 0.2% BSA and 0.05% Tween 20. TMB was used as a substrate.
Abscissa: protein concentration, ng/ml. Ordinate: optical density at 450 nm (SEM from 3 independent measurements).

The following examples are presented to illustrate the present invention.

Example 1.

Design of recombinant plasmids encoding the synthesis of oncoproteins E7 HPV-16 and E7 HPV-18

The E7 gene was isolated from cervical bioptats of patients with diagnosed cervical dysplasias. Clinical samples were examined for HPV using the polymerase chain reaction (PCR). The samples containing HPV DNA (types 16 and 18) were selected for further analysis. The E7 HPV-16 and -18 genes (303 bp –HPV-16 and 324 bp-HPV-18) were amplified by PCR using gene-specific primers and cloned in the EcoRI-BamHI sites of the plasmid pBlueescipt SK(+) (Stratagene). Analysis of oligonucleotide sequences of the cloned genes revealed their complete correspondence to the GenBank data (e.g., AF125673).

The next step included incorporation of translation termination sequences into the 3'-terminal sequence of the structural fragment of the above genes. Fig. 2 discloses structure of the plasmid.

pHE716 encodes the hybrid protein e716: Met(His)6-GluPhelle-E716-GlySer (111 residues., 12500 Da, pI 4,6). This protein is characterized by abnormal mobility (around 21 kDa) during SDS-PAGE electrophoresis according to Laemmli what is consistent with the literary data.

pHE718 encodes the hybrid protein e718: Met(His)6-GluPheSer-E718-GlySer (117 residues, 13500 D, pl 5,4).

BL21(DE3) was used to study gene expression. Induction and disintegration of cells were performed using standard procedures. After ultrasonication, e716 and e718 were detected in the soluble cellular protein fraction; therefore, the chromatography on Ni-IIIA-agarose was carried out under native conditions in the absence of urea (Fig. 3).

Example 2

Preparation of murine monoclonal antibodies to the recombinant proteins E7 HPV16 and HPV18

Female Balb/c mice (16-18 g) were immunized into the foot pads with highly purified E7 HPV16 and E7 HPV18 prepared from recombinant *E.coli* lysates by one-step metal-chelating chromatography. The immunization was performed twice with a 2-week interval.

Protein content per one immunization dose was 20 μg. The protein was injected as a suspension containing an equal volume of complete (1st immunization) or incomplete (2nd immunization) Freund's adjuvant.

On day 4 after the 2nd immunization, the lymphocytes from the popliteal nodes were fused to myeloma cells (Sp 2/0 –Ag14) using PEG 4000. The hybridomas were collected on a selective medium (HAT) and screened in indirect EIA after which positive cultures were cloned twice using the limited dilutions method. Prior to EIA, the recombinant proteins (E7 HPV16 and E7 HPV18) were adsorbed from the solution (2 μg/ml) on polystyrene plates and cultural supernatants (1 h, 37°C) were applied without dilution. The monoclonal antibodies bound to the immobilized antigen were identified using goat antibodies against murine IgG (H+L) conjugated to peroxidase after incubation for 1 h at 37°C. A solution containing TMB and hydrogen peroxide was used as a substrate. Optical density was measured at 450 nm. Two groups of hybridomas to protein E7 HPV16 were obtained, viz., 716-281, 716-288 (IgG2b) and 716-321, 716-325, 716-332, 716-343 (IgG2a), which reacted with E7 HPV16 and HPV18 in indirect EIA with equal efficiency.

Data presented in Table 1 have shown that protein E7 can be regarded as a reliable oncomarker of cervical dysplasias.

Table 1. The values of the optical densities of immobilized proteins E7 HPV 16 and E7 HPV 18 (450 nm) obtained after titration of monoclonal antibodies (data from indirect EIA).

Conc. of MoAb ng/ml	Adsorption of E7 HPV16						Adsorption of E7 HPV18					
	716-281	716-288	716-321	716-325	716-332	716-343	716-281	716-288	716-321	716-325	716-332	716-343
100	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2
30	1.777	1.515	1.382	1.131	1.393	1.737	1.807	1.709	>2	>2	>2	>2
10	0.947	0.705	0.462	0.375	0.473	0.652	1.010	0.939	1.331	1.194	1.350	1.672
3	0.398	0.301	0.160	0.117	0.168	0.265	0.445	0.412	0.508	0.425	0.526	0.839
1	0.154	0.091	0.047	0.030	0.045	0.003	0.176	0.170	0.176	0.145	0.190	0.365
0	0.017	0.007	0.013	- 0.008	- 0.012	0.0	0.029	0.026	0.021	0.020	0.025	0.028

Example 3

Optimization of conditions for immunoenzymatic analysis aimed at quantitation of proteins E7 HPV16 and E7 HPV18 using monoclonal antibodies

MoAb to E7 HPV16 were isolated from the ascitic fluid by affinity chromatography on G-Sepharose (> 95% purity) and labelled with horseradish peroxidase using the periodate method. All possible MoAb combinations were screened pairwise for quantitation of E7 HPV16 using sandwich EIA. To this end, each MoAb was immobilized on polystyrene plates to which seven twofold dilutions of E7 HPV16 (0.039-5.0 ng/ml) were added. The immune complexes formed were developed using all peroxidase MoAb conjugates (TMB was used as a substrate). All of the MoAb pairs were able to form the triple complexes (sandwiches) "immobilized antibody – E7 – conjugate", which points to the oligomerism of the purified protein E7 HPV16 in solution even in the concentration range of $10^{-12} – 10^{-9}$M . Although all MoAb combinations were active in sandwich - EIA test, the pairs consisting of the same antibodies or of antibodies belonging to the same group were less sensitive than MoAb pairs from different groups as could be judged from the values of optical densities at a fixed concentration of E7 HPV16 and the slope of the calibration curve. Owing to their high sensitivity and lack of background effects, MoAb 716-281 were used for coating of plates; MoAb 716-332 was used as a peroxidase conjugate. The conditions for quantitative analysis of E7 HPV16 with this MoAb pair were optimized by varying the concentration of the antibodies to be absorbed, dilution of conjugate, composition of buffer solutions as well as time and temperature for all stages of sandwich-IEA. A calibration curve for quantitation of E7 HPV16 under optimum conditions is shown in Fig.4. As can be seen, the calibration curve is virtually linear at antigen concentrations of 0.039-5.0 ng/ml, is characterized by a complete lack of background effects and has a detection limit for E7 HPV type 16 – 40 pg/ml, which is a crucial factor in determination of expression of E7 HPV16 in transfected cells and clinical samples.

Example 4

Measurement of oncoprotein E7 in cervical samples

Cervical swabs were placed into 1 ml of saline, freezed-thawed thrice and centrifuged in a microcentrifuge (Eppendorf) for 10 min at 10 000 rpm. The supernatant was diluted twice with PBS-AT (PBS, 0,2% BSA, 0,1% Tween) after which a 200-µl aliquot of the

solution was placed into a titration well containing adsorbed monoclonal antibodies against E7-16 and titrated by two- tops dilution per 4 wells. Purified recombinant oncoprotein E7 (type 16) used as a standard was titrated in the same plate (4 to 0.062 ng/ml). After 1-h incubation and washing, a peroxidase-labelled conjugate of monoclonal antibodies to E7-16 was added to the well, incubated for 1 h and developed with TMB. Optical density was measured on a Multiscan at 450 nm. The concentration of E7 in the sample was determined by the value of optical density of the standard cure.

Cervical samples from 28 healthy patients were used as controls. In all cases studied, the E7 test was negative.

Table 2. Determination of oncoprotein E7 type 16 in cervical samples

Number of patient	Anti E7 IgG	E7-16	Total protein OD 1/20	PCR diagnosis	Diagnosis
P1	0.678 0.418	0.1 ng/ml	0.084	HPV 16, 18	CIN III
P2	0.466 0.317	2.2 ng/ml	0.151	HPV 31	CIN I-II
P3	0.232 0.127	0.8 ng/ml	0.152	HPV High oncogenic risk	CIN II-III
P4	0.337 0.158	3.5 ng/ml	0.228		CIN III, suspected cervical carcinoma
P5	0.268	10 ng/ml	0.041	HPV 16, 18	CIN III, suspected cervical carcinoma
P6	0.422 0.202	1 ng/ml	0.058	HPV 16, 18	CIN II
P7	0.215	0.06 ng/ml	0.174	HPV 16, 18	CIN II-III
P8	0.357 0.164	0.3 ng/ml	0.089	HPV 16	CIN I-II
P9	0.412 0.197	1.6 ng/ml	0.096	HPV 18	CIN II-III

P10	0.335 0.130	0.9 ng/ml	0.165	HPV 16	CIN III, suspected cervical carcinoma
P11	0.280	1.2 ng/ml	0.180	HPV 16	CIN II
P12	0.554 0.233	0.8 ng/ml	0.220	HPV 16	CIN II-III
P13	0.443	0.8 ng/ml	0.097	HPV 16	CIN I-II
P14	0.214	1.8 ng/ml	0.162	HPV 16	CIN II

The method of double antibody "sandwich" based on using monoclonal antibodies types 716-281 and 716-332 with enzyme label for quantitative definition of oncoprotein E7 HPV16 in clinical samples possesses a number of conclusive advantages than other methods, such as immunohistochemical, a Western blotting, flow cytofluorimetry etc.;

1. High specificity and absence of a background due to simultaneous linkage of two monoclonal antibodies with different epitopes of target antigen E7 HPV16.
2. High sensitivity of detection level 40 pg/ml due of using big surplus enzyme - marked monoclonal antibodies and modern chromogenic substrates, for example TMB.
3. Extended range of detection from 40 pg/ml up to 50 ng/ml that allows to measure quantitatively concentration of E7 HPV16 with the minimal number of test cultivations.
4. Simple procedure of preparing clinical samples without sample fixing procedure and lavages.
5. Opportunity of simultaneous definition of oncoproteins E7 HPV types 16 and HPV types 18 due of crossing interaction of both types of monoclonal antibodies.
6. Opportunity of screening of a numerous samples and each patient's treatment efficiency monitoring due to highly standardized procedure of collecting and analyzing of clinical cervical samples.

CLAIMS

1. *In vitro* diagnosis method for early detection of cervical dysplasias and cervical cancer, associated with human papilloma viruses characterized in that by using two types - a couple of enzyme-labeled monoclonal antibodies able to recognize different antigenic determinants of oncoprotein E7 HPV (types 16 and 18) identification and quantity definition of oncoprotein E7 HPV (types 16 and 18), which is considered a biochemical marker of malignization of epithelial cells within high sensitive detection range 40 pg/ml - 50 ng/ml provides a reliable proof of the primary stage of neoplastic transformation of cervical cells.

2. A method according to claim 1, wherein the couple of enzyme-labeled monoclonal antibodies are type 716-281 and type 716-332.

3. A method according to claim 1, wherein due to large excess of enzyme-labeled monoclonal antibodies the range of sensitiveness for detection oncoprotein E7 HPV types 16 and 18 is extended from 40 pg/ml to 50 ng/ml.

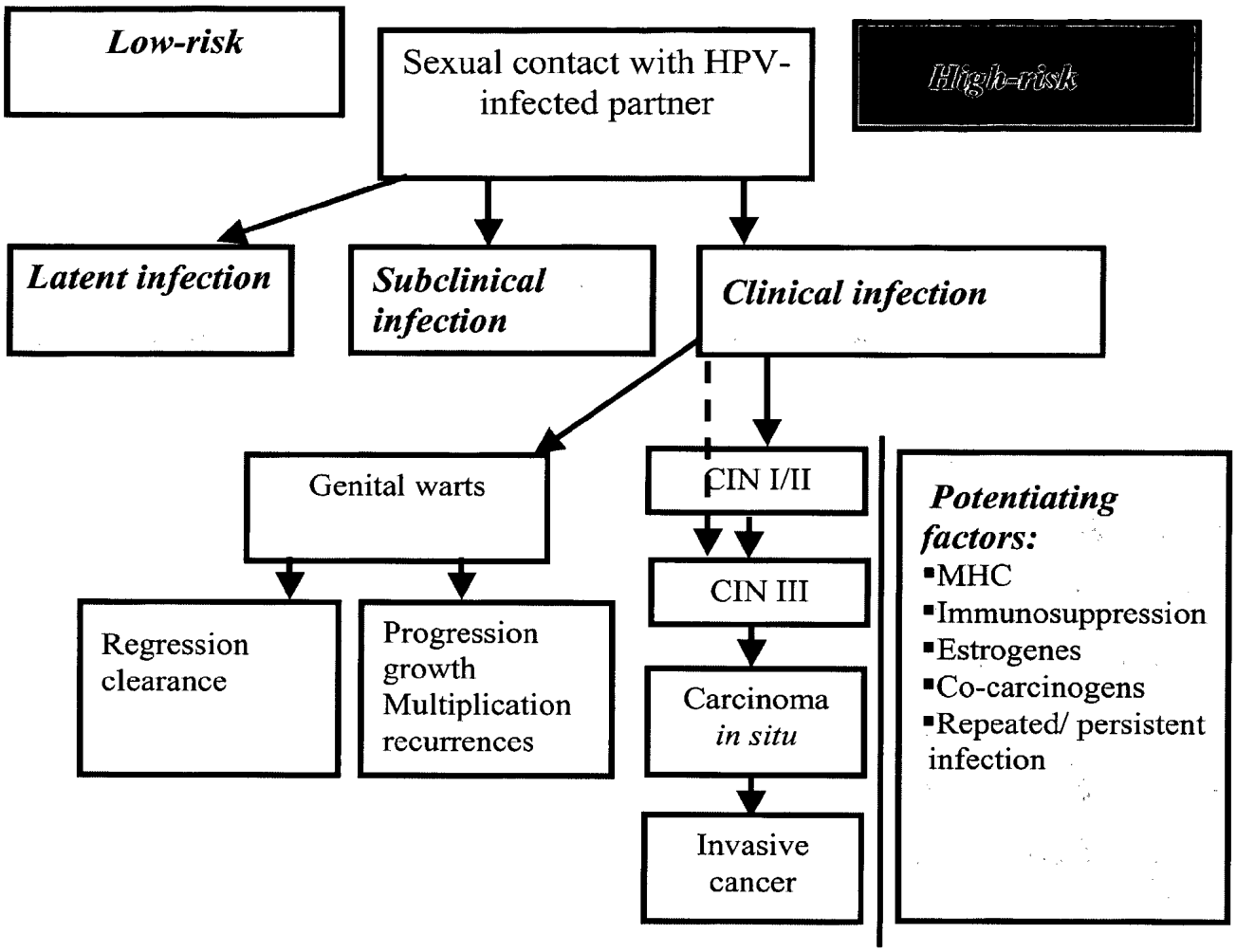
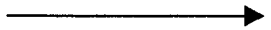

Low-risk
Sexual contact with HPV-infected partner
High-risk
Latent infection
Subclinical infection
Clinical infection
Genital warts
CIN I/II
CIN III
Carcinoma in situ
Invasive cancer
Regression clearance
Progression growth Multiplication recurrences
Potentiating factors:
MHC
Immunosuppression
Estrogenes
Co-carcinogens
Repeated/ persistent infection

Fig. 1

T7 promoter



TAATACGACTCACTATAGGGAGACCACAACGGTTTCCCTCTAGAAATAATTTTGTTTAACT

TTAAGAAGGAGATATACATATGcatcaccatcaccatcacGAATTC - E7 gene HPV16(18) - GGATCC

rbs *Nde I* His-Tag *Eco*RI *Bam*HI

TAATTAGCTGAAAGCTT

Term *Hin*DIII

Fig. 2

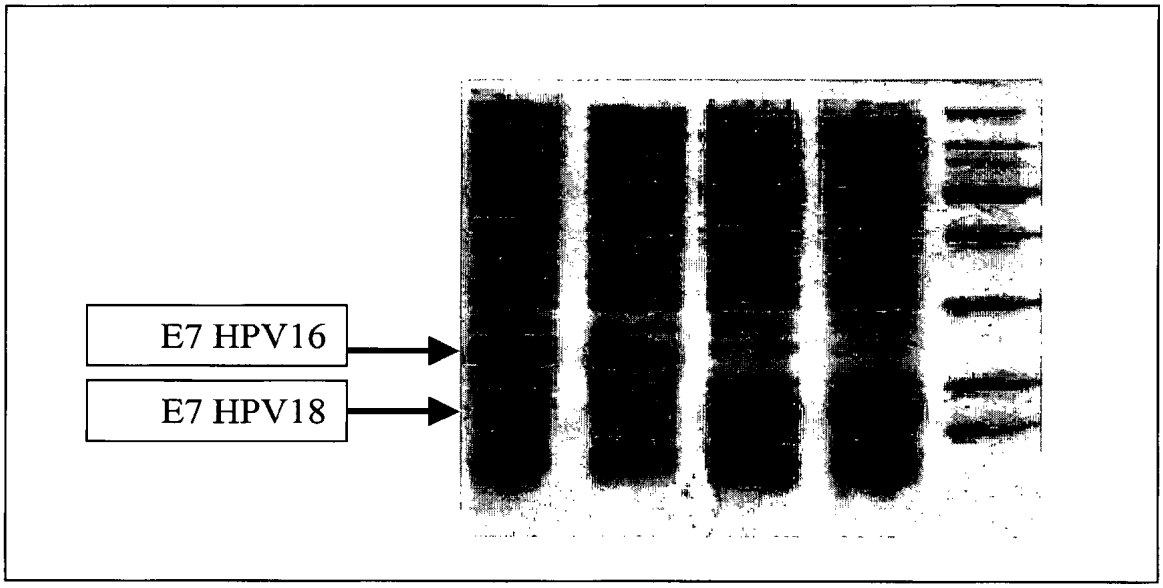


Fig. 3

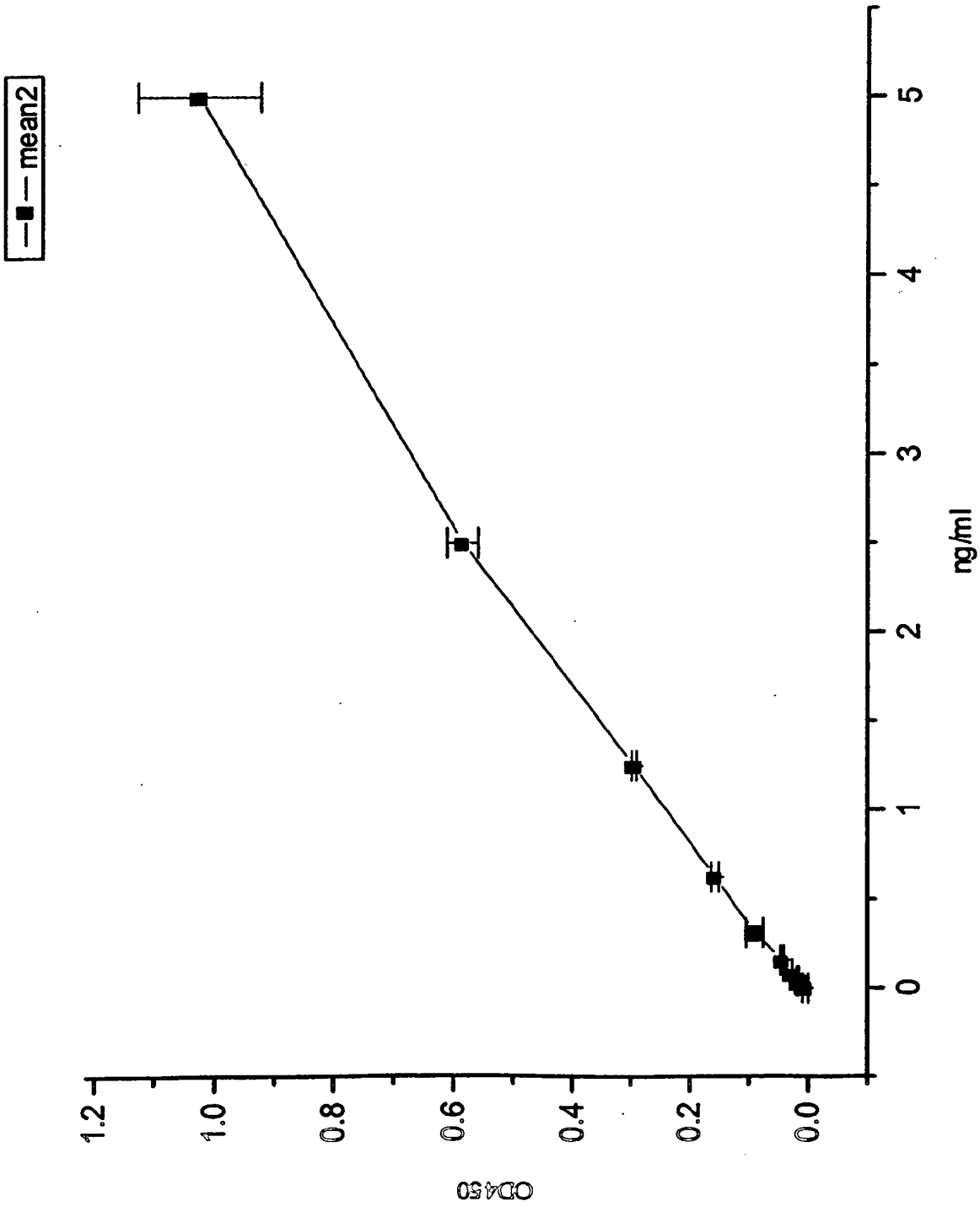


Fig. 4

INTERNATIONAL SEARCH REPORT

International Application No
PCT/LT 03/00004

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N33/574

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, BIOSIS, EMBASE, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95/15497 A (UNIV QUEENSLAND ; FRAZER IAN HECTOR (AU); DUNN LINDA ANN (AU); TINDLE) 8 June 1995 (1995-06-08) the whole document	1-3
X	SELVEY L A ET AL: "AN ELISA CAPTURE ASSAY FOR THE E7 TRANSFORMING PROTEINS OF HPV16 AND HPV18" JOURNAL OF VIROLOGICAL METHODS, AMSTERDAM, NL, vol. 37, no. 2, 1992, pages 119-127, XP001091302 ISSN: 0166-0934 the whole document	1-3

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
24 August 2004	06/09/2004

Name and mailing address of the ISA	Authorized officer
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LONARDO DI A ET AL: "Egg yolk antibodies against the E7 oncogenic protein of human papillomavirus type 16" ARCHIVES OF VIROLOGY, NEW YORK, NY, US, vol. 146, no. 1, 2001, pages 117-125, XP002253651 ISSN: 0304-8608 the whole document	1-3
X	WO 01/54713 A (MUSC FOUNDATION FOR RES DEV ; MATHUR RAJESH S (US); MATHUR SUBBI P (US) 2 August 2001 (2001-08-02) cited in the application the whole document	1-3
X	RESEARCH DIAGNOSTICS INC: "HPV 16 Late I Protein & E7 protein antibodies from Research Diagnostics Inc" INTERNET DOCUMENT, 'Online! 20 January 2003 (2003-01-20), XP002293548 Retrieved from the Internet: URL:http://www.researchd.com/tumorabs/cb1402.htm> the whole document	1-3
L	-& WEB.ARCHIVE.ORG: "Internet Archive Wayback Machine" INTERNET DOCUMENT, 'Online! 24 August 2004 (2004-08-24), XP002293606 Retrieved from the Internet: URL:http://web.archive.org/web/*/http://www.researchd.com/tumorabs/cb1402.htm>	
P,X	WO 03/080669 A (AMYNON BIOTECH GMBH ; JANSEN-DUERR PIDDER (AT); MUELLER-HOLZNER ELISAB) 2 October 2003 (2003-10-02) the whole document	1-3

3

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/LT 03/00004

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9515497 A	08-06-1995	AU 1103595 A	19-06-1995
		WO 9515497 A1	08-06-1995
WO 0154713 A	02-08-2001	AU 3465501 A	07-08-2001
		CA 2398477 A1	02-08-2001
		EP 1253934 A1	06-11-2002
		WO 0154713 A1	02-08-2001
		US 2003017505 A1	23-01-2003
WO 03080669 A	02-10-2003	WO 03080669 A2	02-10-2003